CLINICOPATHOLOGICAL STUDY OF NON IMMUNE THROMBOCYTOPENIA

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CERTIFICATE

This is to certify that this dissertation entitled **"CLINICOPATHOLOGICAL STUDY OF NON – IMMUNE THROMBOCYTOPENIA"** is a bonafide work done by **Dr.P.M.BALAJI,** in partial fulfillment of the requirements of THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY, Chennai for the award of **M.D. Pathology Degree.**

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DECLARATION

I declare that this dissertation entitled **"CLINICOPATHOLOGICAL STUDY OF NON – IMMUNE THROMBOCYTOPENIA"** has been done by me under the guidance and supervision of **Prof. Dr.GEETHA DEVADAS, M.D., D.C.P.** It is submitted in partial fulfillment of the requirements for the award of the M.D. Pathology degree by The Tamilnadu **Dr. M.G.R. Medical University**, Chennai. This has not been submitted by me for the award of any degree or diploma from any other University.

Dr.P.M.BALAJI

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LIST OF ABBREVIATIONS

SLE	Systemic Lupus Erythematosis
AML	Acute Myeloid Leukemia
ALL	Acute Lymphoblastic leukemia
PT	Prothrombin time
APTT	Activated partial thromboplastin time
LDH	Lactate dehydrogenase
DIC	Disseminated intravascular coagulation
CML	Chronic myeloid leukemia
MDS	Myelodysplastic syndrome
ANA	Antinuclear antibody
ACLA	Anti Cardiolipin antibody

CONTENTS

INTRODUCTION

Thrombocytopenia may be defined as a subnormal number of platelets in the circulating blood ie less than 150 x 10^9/L.

It is the most common cause of abnormal bleeding. The causes of thrombocytopenia are diverse but they can be grouped according to the distribution by an immne or non immune mechanism or requestration of platelets.

Pseudothrombocytopenia also should be considered in the differential diagnosis particularly in asymptomatic patients and results from invitro clumping of platelets following blood collection.

The differential diagnosis of thrombocytopenia in adult is broad but is acquired in the vast majority of patients, usually associated with an underlying disease or because of an auto immune process. A large number adult patients are diagnosed incidentally on routine examination careful examination o the blood film is the bes means for narrowing the differential diagnosis.

This prospective study focuses on the analysis of adult patients confirmed with thrombocytopenia using clinical and relevant laboratory parameters to assure at a possible aetiopathogenesis.

AIMS AND OBJECTIVES

1. To evaluate patients with thrombocytopenia presenting to the clinical pathology, medical OP and haematology OP departments of Government General Hospital and Madras Medical College.

2. To evaluate those patients with thrombocytopenia in whom additional pathogenetic mechanisms were suspected.

REVIEW OF LITERATURE

Megakaryopoiesis and Thrombopoiesis

Each day the adult human produces approximately $1x10^{11}$ platelets,which can increase 10 to 20 fold in times of increased demand.

Production of platelets depends on the proliferation and differentiation of haematopoietic stem and progenitor cells to a cell committed to the megakaryocytic lineage, its maturation to a large polyploid megakaryocyte and its final fragmentation into platelets.

A supportive marrow stroma consisting of endothelial and other cells, matrix glyosaminoglycans and a family of protein hormones and cytokines including thrombopoietin,stem cell factor,IL-6,IL-11 and stromal cell derived factor I are the external factors that impact megakaryopoiesis and thrombopoiesis.

The maturation stages of the megakaryocyte include:

Megakaryoblast —Basophilic megakaryocyte —Granular megakaryocyte —

Mature megakaryocyte.

Platelet Production

Platelets are small (2 µm) non nucleated blood cells that play a vital role in hemostasis and are produced in the bone marrow from megakaryocytes[1].Megakaryocytes are descended from pleuripotent hematopoietic progenitors through a bipotential erythroid/megakaryocytic cell.After this stage megakaryoblasts undergo maturation to megakaryocytes stimulated by thrombopoietin and other cytokines and chemokines.Maturation is dependent on transcription factors GATA 1 and GATA 2 together with cofactor FOG1.This maturation is accompanied by **endomitosis** which is **increasing cellular DNA content without proliferation**. During a terminal phase of megakaryocytic differentiation, platelets are produced from cytoplasmic projections known as protoplatelets.Transverse bands of microtubules form along longitudinal bundles of microtubules in the proptoplatelets with breakage at constriction zones,and liberation of newly formed platelets from the tip of the protoplatelet.Upon release from the bone marrow platelets circulate in a **quiescent state** but are **activated rapidly** upon blood vessel injury and play a crucial role in the primary hemostatic response.

Platelet Morphology

Bizzozero (1882) described platelets in mesenteric vessels of rabbits and guinea pigs. He demonstrated their adhesive qualities, participation in thrombosis and role in coagulation of blood. Origin of the platelet from the megakaryocyte was established by the studies of Wright. The platelets in peripheral blood are heterogeneous with respect to size, density, staining characteristics. Their morphology also varies with the methods by which they are examined ,the anticoagulant and isolation method employed and the temperature. In wet preparations, platelets are colourless, moderately refractile bodies that are discoid or elliptical.Under dark field illumination they are translucent and reveal a sharp contour. A few immobile granules are present in the centre of the cell. In polychrome stained smears platelets appear round, oval and rod shaped. Azurophilic granules are seen in a hyaline light blue cytoplasm. The terms granulomere and hyalomere have been applied to the granular and hyaline portions respectively.

Platelet dimensions and variations in disease

Average dimensions of the human platelet is 3.6 ± 0.7µm in diameter and 0.9±0.2 µm in thickness.

Platelet anisocytosis and macrocytosis have been studied in stained smears when platelet production is accelerated. Platelets that are 2.5 µm in diameter or have mean platelet volumes in excess of 13 fl.

(megathrombocytes) constitute less than 10% of population. These megathrombocytes increase in accelerated platelet destruction eg. auto immune thrombocytopenia.Platelets with giant fusion granules have been described in individuals with refractory anemia and preleukemia.Platelet sizing with automatic particle counters have revealed abnormally small platelets in association with aplastic anaemia, sepsis-associated thrombocytopenia and hypersplenism .By contrast large and bizarre platelets are often noted after splenectomy in patients with myelofibrosis, haemorrhagic polycythaemia and polycythaemia vera.Certain forms of thrombocytopenia associated with disordered platelet function are characterized by particularly large giant platelets. In peripheral blood smears platelets may adhere to the periphery of the neutrophils called platelet satellitism. This is also seen as an invitro artifact resulting from Ig E or Ig M platelet agglutinins that are active in anticoagulated blood.

Megakaryocyte

These are precursors of platelets which are exceedingly large cells ranging from 35-160 µm in diameter. They contain an irregular lobed, ring or doughnut shaped nucleus that is made up of dense chromatin that stains dark blue with Wright's stain. The cytoplasm is abundant light blue packed except for a narrow rim at the periphery with fine azurophilic granules.

Variations in size and morphology

When platelet production is accelerated, megakaryocytes increase both in size and number. In various forms of thrombocytopenia caused by accelerated platelet destruction, one may see immature forms that are not present in normal marrow, morphologically abnormal and degenerating megakaryocytes that do not appear to be producing platelets.

Abnormally large hyperlobulated megakaryocytes with thin nuclear chromatin have been described in folic acid and Vitamin B12 deficiency. Numerous morphologically abnormal megakaryocytes often are noted in marrow specimens by biopsy from patients with myelofibrosis. Micro megakaryocytes are common in patients with pre leukemia and chronic myelomononcytic leukemia.

EM appearance of platelets

Electron Microscopy reveals a fuzzy coat (glycocalyx) extending 14 to 20 nm from the platelet surface, which is thought to be composed of membrane glycoproteins,glycolipids,mucopolysaccharides and adsorbed plasma proteins [2] .

Platelets move in an electric field as if they have net negative surface charge. Sialic acid residues attached to proteins and lipids are major contributors to this negative charge[3].Electrostatic repulsion created

by the negative surface charge may help prevent resting platelets from attaching to each other or to negatively charged endothelial cells.

The surface of the platelet has a number of indentations that are an elaborate channel system composed of invaginations of the plasma membrane that extend throughout the platelets.

The contents of the platelet granules can gain access to the outside when the granules fuse with either the plasma membrane or any other region of the open canalicular system. Similarly, glycoprotiens contained within granule membrane can join the plasma membrane after granule fusion with either the plasma membrane or the open canalicular system.

Platelet function

As stated earlier, platelets ordinarily circulate in the blood stream in a quiescent state but undergo explosive activation following damage to the vessel wall leading to rapid formation of a platelet aggregate or vascular plug and occlusion at the site of damage. Platelets are therefore enriched with signalling proteins and surface receptors that enable them to achieve a rapid response.[4, 5]

The responses associated with platelet activation and their roles in haemostasis is discussed below.

Adhesion

The initiating event following vascular damage is platelet adhesion to exposed subendothelial matrix proteins. The platelet glycoprotein receptors which mediate adhesion are dependent on rate of shear. Under the intermediate to high shear found in arterioles, this event is strictly dependent on Von-Willebrand factor VWF and its receptor GP 1b- IX – V complex. Adhesion applies also to recruitment of circulating platelets into the thrombus. The platelet bound VWF that supports these events is derived from plasma and via secretion from platelet α-granules. Adhesion to the growing thrombus is supported by binding of fibrinogen to the Integrin α IIbβ3 a process that is more correctly termed aggregation.

Shape change and spreading

Upon activation platelets become spherical and extend pseudopodia to enable them to attach to other platelets and to the vessel wall. The transition to a sphere increases the optical density. This is mediated by phosphorylation of myosin light chains by elevation of intracellular Ca^{2+} ions which activate myosin light chain kinase or through inhibition of myosin light chain phosphatase.

Adhesion of platelets to a reactive surface such as collagen or fibrinogen is first characterized by transition from a discoid to a more rounded form but this is then followed by formation of filopodia which grow from the periphery of the cell and lamellipodia which fill in the area

between adjacent filopodia. As the platelet flattens and spreads, granules and organelles are squeezed into the centre of the cell resulting in a characteristic fried egg appearance.Granules are secreted from the centre of the cell directly into the surface open canalicular system before release into the surrounding medium.These dramatic changes in morphology are brought about by a powerful severing and reassembly of actin cytoskeleton through regulation of a number of actin regulatory proteins.

Aggregation

This involves crosslinking of platelets through binding of fibrinogen or other ligands such as VWF to Integrin αIIbβ3 on adjacent cells. The major platelet Integrin GP IIb –IIIa forms a bridge between the platelet cytoskeleton and the polymerized fibrin that is generated by the coagulation cascade [6].

Upon platelet activation,αIIbβ3 undergoes conformational change that increases the affinity for the fibrinogen,VWF, fibronectin and CD40 ligand. Binding of fibrinogen to αIIbβ3 reinforces platelet activation. Elevation of Ca^{2+} is required for rapid activation of αIIbβ3 and plays an essential role in thrombus formation.

Secretion

Platelets contain three main types of storage granules. Dense, α granules and lysosymes each of which rapidly release their contents upon activation.α granules are most numerous- about 80/Platelet and contain a

rich diversity of proteins and membrane receptors that support many processes in haemostasis. Dense granules contain high levels of small molecules that support platelet activation and mediate vasoconstriction. Lysosymes also release their contents on activation. Platelet dense granules contain ADP and ATP, 5HT and Ca^{2+} ions. The rapid release of ADP from dense granules plays a major positive feedback role in promoting platelet activation. Platelet α granules contain fibrinogen, VWF, Integrin αIIbβ3. P-Selectin in the α granule membrane stabilizes the thrombus. Factor V,VIII,protein- S,Chemokines like Platelet factor 4 and RANTES, which attract circulating leukocytes, are also found in the α granules.

Recently a component of α granules, the vitamin K dependent protein GAS 6 plays a major role on supporting platelet aggregation.

Phospholipase A_2 and Thromboxane A_2 are the two major platelet positive feedback agonists. Platelet derived micro particles are generated during platelet activation and reinforces thrombus formation. Platelets are the force generating components of clot retraction.

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Approach to a patient with Thrombocytopenia

Definition: Thrombocytopenia is defined as a platelet count below the normal range of 150 – 400 x 10^9/L .

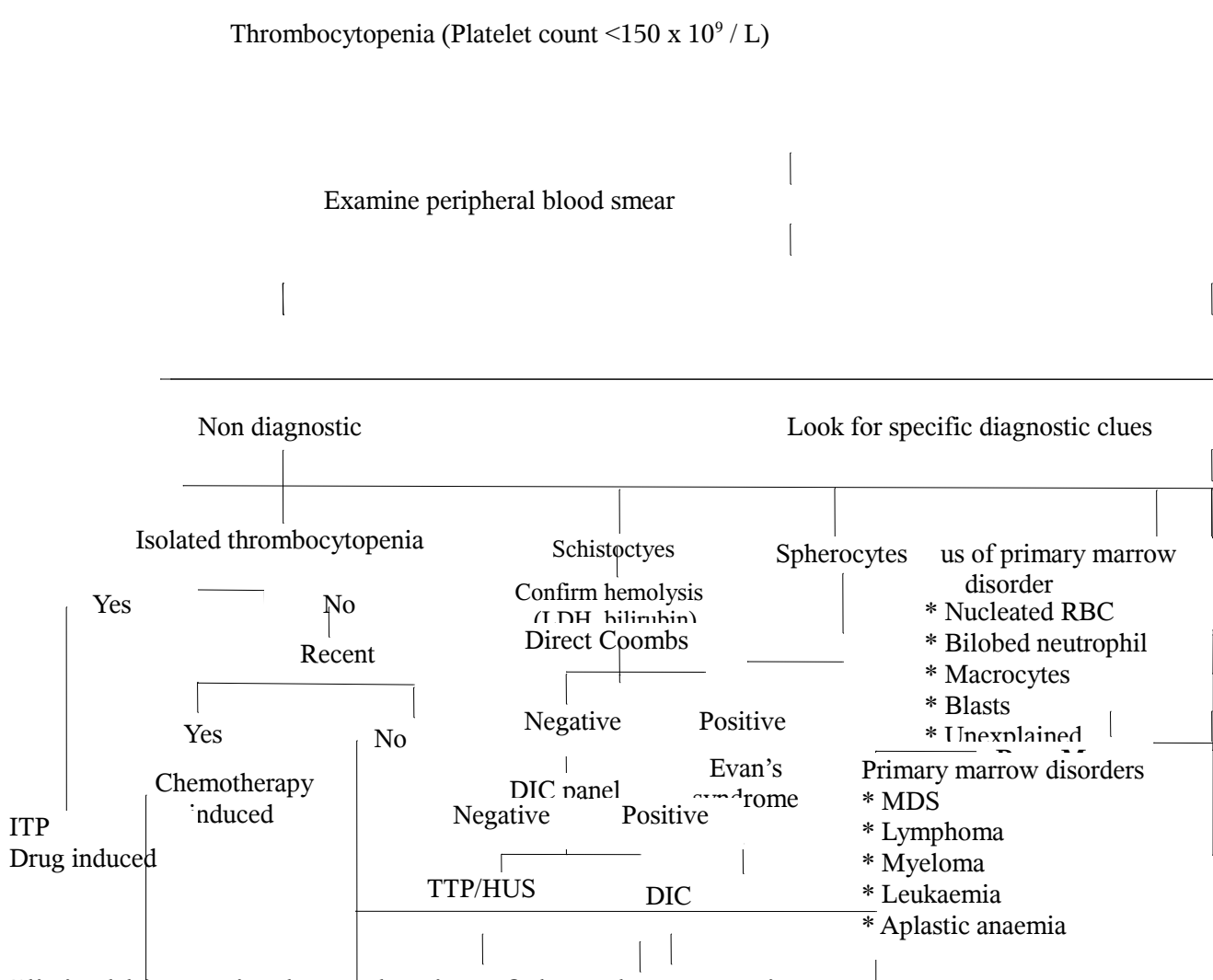
It is the most common cause of abnormal bleeding. Despite the number and diversity of disorders that may be associated aetiologically, thrombocytopenia results from mainly four processes – artifactual thrombocytopenia,deficient platelet production,accelerated platelet destruction and abnormal distribution or pooling of platelets within the body.

When faced with an asymptomatic patient with a low platelet count, the possibility of artifactual or pseudothrombocytopenia has to be excluded. Artifactual thrombocytopenia or falsely low platelet counts occurs exvivo when platelets are not counted accurately. Inaccurate counting may occur in the presence of giant platelets or with platelet satellitism.[7,8] The most common cause of artifactual thrombocytopenia is platelet clumping. This is caused by anticoagulant dependent platelet agglutinins that are immunoglobulins IgG,A,M. It is most common with EDTA anti coagulant [9] as evidenced by the studies of Gowland et al. The presence of platelet clumps on examination of the peripheral blood smear and normal platelet count using citrated blood confirms pseudo thrombocytopenia as the cause. Electronic cell counters sometimes show false low counts due to giant platelets. If repeated samples show low

counts which do not correlate with the clinical parameters, it is mandatory to confirm platelet count by manual counting methods and to confirm the count by a well stained peripheral smear.

If thrombocytopenia is confirmed, a stepwise evaluation should be undertaken to assess the causes [10] .

ALGORITHM FOR WORKUP OF THROMBOCYTOPENIA



Clinical history in the evaluation of thrombocytopenia

A detailed clinical history is very helpful in arriving at a possible cause of thrombocytopenia and should include the following:

Bleeding — age at onset — Congenital thrombocytopenia may present as a delayed onset

Acquired eg CMV infection may present at birth.

Acute

Chronic

Spontaneous

Trauma / surgical intervention

	Predominantly mucocutaneous	
	Deep seated / intramuscular	
	Associated symptoms	
	* Fever	
	* Drugs	
	* Arthralgia / arthritis	
	* Jaundice	
	* Pallor	
	Transfusions given	Platelet alone other components as well

Note: In females, a detailed menstrual and obstetric history must be obtained.

Physical Examination

This must include evaluation of pallor, fever, lymphadenopathy, jaundice, sternal tenderness, gum hyperplasia, hepatomegaly, splenomegaly, ascites and exanthematous rash for viral/vasculitic aetiology.

Laboratory investigations

The results of the complete blood count and review of the peripheral blood smear are critical components in the initial evaluation of the thrombocytopenic patient.[11]

Platelet count

Since the introduction of automated cell counters,the number of platelet counts performed manually have decreased significantly.[12,5] The accepted normal range of the platelet count is 150-400x10^9/L.

Mean platelet volume [MPV]

This is obtained using new generation cell counters. The normal values of MPV depends on the platelet count. To determine if the platelet values are normal the platelet count and the MPV should be related to a normogram with a combination of high, low and normal platelet count and a high, low and normal MPV.A high MPV has been correlated with myeloproliferative disorders and thalassemia and a low MPV is

associated with hypoplasia and cytotoxic drugs. For patients with thrombocytopenia, a high MPV indicates increased platelet destruction and a low MPV suggests decreased platelet production. Normal MPV ranges are approximately 7-11 fl.

PLATELET DISTRIBUTION WIDTH[PDW]

PDW is a measure of the dispersion of the platelet sizes.In patients with rapid turnover,the platelets will in general be larger due to the larger size of the newly produced platelets and their PDW will be increased due to mixture of large and small platelets. True congenital macrothrombocytopenias usually have uniformly large platelets with a very high MPV and normal PDW.

BONE MARROW EXAMINATION

Bone marrow examination and trephine biopsy can be performed to evaluate etiology of thrombocytopenia.In a thrombocytopenic patient, when no other reason for low platelet counts can be determined, the bone marrow examination is useful for determining the presence of megakaryocytes. Absence indicates dysfunctional marrow while increased numbers suggest peripheral destruction with attempted bone marrow decompensation. Bone marrow examination can also detect myelophthisic disorders such as acute leukemia or metastatic malignancy.

COAGULATION PROFILE

Prothrombin time, activated partial thromboplastin time, plasma fibrinogen levels and D-dimer evaluation is done to exclude an associated coagulopathy.

Other ancillary investigations relevant in the work up of thrombocytopenia include Coomb's test, serum LDH levels, serum uric acid levels, renal function tests, liver function tests and confirmatory tests pertaining to specific cases.

PATHOPHYSIOLOGICAL CLASSIFICATION OF THROMBOCYTOPENIA

I.DECREASED PRODUCTION:

MARROW INFILTRATIVE DISORDERS:

- Malignant disorders — haematological
  - Lymphoma
  - Leukemia
  - myeloma
- Metastatic
- Non neoplastic
  - Secondary myelofibrosis — auto immune granulomatous storage disorder
  - Infections — Lymphohistiocytic infiltration in CMV

INEFFECTIVE HAEMATOPOESIS

- Myelodysplastic syndromes
- Deficiency anaemias-B_{12} deficiency

HYPOPLASTIC DISORDERS

Aplastic anaemia

- Congenital
- Acquired
  - * Idiopathic
  - * Drugs
  - * Chemicals
  - * Radiation
  - * Infection

4) HERIDITARY THROMBOCYTOPENIAS

II. INCREASED DESTRUCTION

1. **Immune:** Isolated presentation

- Immune thrombocytopenia purpura

As a part of Connective tissue disease

- Heparin induced thrombocytopenia
- Drug induced antibodies
- HIV
- Post transfusion
- Connective tissue diseases

2. **Non Immune:**

- DIC
- Sepsis
- Cardiac valves
- TTP/HUS
- Kasabach Merrit Syndrome
- HIV

- Post transfusion purpura

III. SEQUESTRATION:

- Hypersplenism
- Macrophage activation syndromes

DECREASED PRODUCTION DUE TO MARROW INFILTRATION

Thrombocytopenia in hematological malignancies are clonal malignant proliferations of the hematopoietic stem cell that is characterized by the accumulation of blasts principally in the marrow at the cost of impaired production of normal blood cells .[13,14] Non hematological malignancies commonly implicated are carcinomas of lung, breast and prostate. Marrow trephine biopsy is preferred in the detection of these tumours.

Non neoplastic conditions causing secondary myelofibrosis and consequent cytopenias including thrombocytopenia are autoimmune disorders namely SLE, Rheumatoid arthritis and Sjogrens syndrome [15], granulomatous disorders of marrow including viral, fungal, bacterial, rickettsial and toxoplasmosis[16] and storage disorders including Gaucher's disease and Niemann Pick disease.

Common viral infections include HIV infection ,[17] most common cause in North America and cytomegalovirus infection causing lymphohistiocytic infiltration of the marrow.

INEFFECTIVE HAEMATOPOIESIS

Marrow hypoplasia due to ineffective haematopoesis include myelodysplastic syndromes and deficiency anaemias notably B12. Myelodysplastic syndromes are clonal stem cell disorders characterized by blood cytopenias in combination with a hypercellular marrow that often exhibits dysplastic changes in any of the three haematopoietic lineages. [18] Thrombocytopenia is present in approximately 50% of patients and occurs in conjunction with other cytopenias.[19]

In megaloblastic anaemia due to Vit B12 deficiency, there is a moderate reduction of platelet count rarely $< 40 \times 10^9$ / L. Thrombocytopenia is due to ineffective megakaryopoiesis.

HYPOPLASTIC ANAEMIA

Hypoplastic disorders affecting marrow include aplastic anaemia which is a clinical syndrome that results from marked dimunition of marrow blood cell production. The decreased production results in reticulocytopenia, anaemia, granulocytopenia, monocytopenia and thrombocytopenia. In paroxysmal nocturnal hemoglobinuria,

thrombocytopenia can be accompanied by thrombotic manifestations due to activation of platelet by complement.

HERIDITARY THROMBOCYTOPENIA

These disorders are common in children and are due to congenital defects in platelet production. Bernard Soulier syndrome is a congenital deficiency of platelet GP 1b α / 1bβ/ 1X / V receptor.

THROMBOCYTOPENIA DUE TO PERIPHERAL DESTRUCTION OF PLATELETS

Immune Thrombocytopenia

Immune thrombocytopenia is a relatively common disease in adults. A Danish study noted incidence rate of 2.68 per 100000[20]. ITP is an autoimmune condition caused by antiplatelet antibodies which result in decreased platelet survival. The antibodies are frequently IgG in nature and directed against platelet antigens - GP II b / III a and GP 1b / 1X complexes. The spleen is the major site of platelet destruction. Frequently patients are young adult females. Severe thrombocytopenia typically presents without anaemia or leukopenia.

Heparin induced thrombocytopenia

Heparin induced thrombocytopenia (HIT) is a common cause of drug induced thrombocytopenia in hospitalized patients. Two types of HIT have been described.

Type I HIT is a modest transient decrease is platelet count that occurs within the first 2-3 days after heparin initiation and returns to normal spontaneously even with continuation of heparin.

Type II HIT is seen in 0.3 – 5 % of patients treated with unfractioned heparin [21]. It is caused by antibodies against platelet factor 4 – heparin complex. Thrombocytopenia sets is usually 2 weeks or more after first exposure. Subsequent exposure results in thrombocytopenia immediately or after few days.

Post transfusion purpura

Post transfusion purpura is a rare complication of blood transfusion seen mostly in women. It presents as severe thrombocytopenia 5 – 10days after red cells or platelet transfusion. This is an alloimmune complication that occurs in patients who have developed antiplatelet antibodies from prior transfusion or pregnancy [27].

NON IMMUNE CAUSES OF PLATELET DESTRUCTION

Disseminated intravascular coagulation (D1C)

DIC is a systemic process caused by pathologic thrombin generation characterized clinically by thrombosis and bleeding.

Etiological factors include amniotic fluid embolism, abruptio placentae, trauma, snake bite, leukemias (AML-M_3) and adenocarcinoma.

Thrombocytopenia is almost universal in patients with DIC due to activation of the clotting mechanism [22].

Prosthetic cardiac valves

RBCs on contact with prosthetic valves are constantly subject to damage. The degree of anaemia is proportional to the compensatory mechanisms. A good reticulocyte response ensures adequate haemoglobin values, nevertheless fragmented RBC can be a chronic trigger for a sub-clinical microangiopathy resulting in chronic consumption of platelets.

Thrombotic thrombocytopenic purpura / Haemolytic uremic syndrome (TTP/HUS)

TTP / HUS is a relatively uncommon life threatening cause of thrombocytopenia[23]. Classic diagnostic pentad of TTP includes.

1. Microangropathic hemolytic anaemia
2. Thrombocytopenia
3. Renal insufficiency
4. Fever
5. Mental status changes

The presence of thrombocytopenia and schistocytes > 1 / HPF on smear is commonly seen. Serum LDH is increased. Thrombocytopenia

can be severe with a median platelet count of 20000. A diagnosis of TTP – HUS should be considered is a patient with thrombocytopenia and evidence of microangiopathy [24].

Kasabach Merrit Syndrome

It is a rare cause of destructive thrombocytopenia with cavernous haemangimas associated with microangiopathy, haemolytic anaemia.

SEQUESTRATION

1) Hypersplenism

Splenomegaly from any cause including neoplastic,congestive, infiltrative and infections result in sequestration of blood elements leading to cytopenias. The cardinal features of hypersplenism are a) splenomegaly b) reduced levels of one or more blood elements in circulation associated with increased precursors. c) Correction of cytopenia afte splenectomy [28].

Splenomegaly is almost always secondary to other disorders most commonly cirrhosis with portal hypertension. Thrombocytopenia is usually of moderate severity rarely below 40000 / µl. Platelet transfusions are not effective as transfused platelets are also sequestered in the spleen.

2) Macrophage activation syndromes

Systemic macrophage activation syndrome can occur in response to a number of different stimuli. Viral infections are best known, but bacterial infections, rheumatological disorders, intravenous alimentation and multiple organ failure are among other causes. This appears to be due to lymphocyte / NK cell driven macrophage stimulation that leads to disseminated overactivity of the macrophages throughout the body[25]. An increase in the number and size of endogenous macrophages with or without haemophagocytosis, is a feature of this condition, best seen in the marrow, but also in the spleen, liver and lymphnodes. There may be severe functional effects of the cytokine storm with bone marrow depression, hepatomegaly and increased heptocellular enzymes and effects on the clotting cascade[26]. The condition abates when the inciting condition is treated or disappears or may at times be fatal.

Pregnancy and thrombocytopenia

Thrombocytopenia in pregnancy can occur in the following settings :

a) Gestational (Incidental)

b) Immune thrombocytopenic purpura.

c) HELLP syndrome (hemolysis, elevated liver enzymes and low platelets)

d) Antiphospholipid syndrome and SLE.

e) Hypertensive disorders.

Most common is incidental thrombocytopenia (upto 75% of cases). Hypertensive disorders account for 20% of pregnancy associated thrombocytopenia [29]. Subset of these patients have a syndrome called HELLP[30].

MATERIALS AND METHODS

This prospective study was conducted in the Goschen Institute of Pathology, Madras Medical College from 2007-2009. The study included

33 subjects who presented to the clinical pathology department and medical OP departments of Madras Medical College.

Inclusion Criteria

Patients presenting to the clinical pathology and medical OP departments who were found to have thrombocytopenia, defined as a platelet count less than 150 x 10^9 / L in an automated counter and confirmed by peripheral smear examination.

Exclusion Criteria

Patients who, after detailed clinical assessment and laboratory tests were diagnosed to have an immunological basis for thrombocytopenia.

A detailed history was elicited from each patient included in the study population.

1) Patient identity details

These include name, age, sex, hospital number, address and unit.

2) Chief presenting complaints and duration.

3) History of present illness - The relevant symptomatology associated with the chief complaint.

4) Past history:

   a) H/O chemotherapy / radiotherapy

   b) malignancies

   c) previous surgery or blood transfusion

   d) drugs ingested in the past with emphasis on intake of indigenous medicines

   e) previous bleeding tendencies and site of bleeding

   f) chronic ailments like diabetes mellitus, systemic hypertension, bronchial asthma, ischaemic heart discase, pulmonary tuberculosis, epilepsy.

5) Personal history

   a) alcohol intake-quantity, duration

   b) Tobacco / paan intake and duration

   c) Extra marital sexual contact

d) Menstrual history (in women) : age at menarche, duration of the menstrual flow, regularity and duration of the menstrual cycle, age at menopause.

e) Obstetric history (in women): marital status, number of children, abortions.

General examination

This included assessment of vital parameters , nutritional status, pallor, icterus, clubbing, pedal oedema, evidence of bleeding, sternal tenderness, lymphadenopathy.

Other features were assessed if warranted on the basis of provisional diagnosis.

Systemic examination

Assessment included examination of cardiovascular, respiratory, central nervous system and abdomen.

A provisional clinical diagnosis was made with differential diagnosis and necessary laboratory tests were performed which included the mandatory tests as per the protocol besides other relevant specific tests.

Laboratory tests

Peripheral venous blood obtained from antecubital venipuncture was used. Appropriate amounts of blood were transferred into tripotassium EDTA vacutainer for complete blood count, sodium citrate 3.2 % for coagulation profile and plain blood in sterile tubes for biochemical analysis. Some blood was preserved at -20^{o}C.

The complete blood count was performed using SYSMEX KX-21 3 part cell counter. The following parameters were included: Total leucocyte count, differential count, haemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width, platelet count, mean platelet volume and platelet distribution width. ESR was measured by Westergren's method. Peripheral blood smear stained by Leishman stain was examined.

Bone marow study

Bone marrow aspirate was obtained from posterior superior iliac crest using 16G bone marrow aspirate needle. Smears were stained with Leishman stain. Bone marrow iron studies were performed in some cases. In relevant cases bone marrow trephine biopsy was performed and H & E stained paraffin sections were examined.

Coagulation profile

Blood with appropriate amount of 3.2 % sodium citrate was used for prothrombin time, activated partial thromboplastin time and fibrinogen estimation. PT, aPTT and fibrinogen was done using coagulatory method in SYSMEX CA-500. D-dimer levels were estimated in select cases.

Biochemical tests

Plain blood was used for assessing liver function, renal function and estimation of serum lactate dehydrogenase.

Liver function tests

The following parameters were assessed

a) Total and direct bilirubin

b) SGOT, SGPT

c) Serum alkaline phosphatase

d) Total protein and albumin

Renal function tests

The following parameters were assessed

a) Urea

b) Creatinine

c) Serum electrolytes

Serum LDH was estimated in all patients.

Ultrasonogram was performed in all cases.

For patients with suspected infections, the following tests were done:

a) Smears for MP, MF and QBC method

b) MSAT for Leptospirosis

c) Dengue IgG and IgM antibodies

d) HIV- I & II antibodies by rapid and ELISA method

e) Blood culture for enteric and non enteric organisms

f) Gram stains

g) Stains for AFB, fungus

h) Sputum examination

i) Rheumatoid factor

j) ANA

k) ACLA

When malignancy was suspected, the following tests were performed.

a) CT scan

b) MRI

c) Fluid analysis for effusions including cytological study.

d) FNAC in cases of palpaple masses. eg. cervical lymphadenopathy.

e) Histopathology to exclude or confirm malignancies.

RESULTS

The total number of cases were analysed and categorized according to the final diagnosis. The distribution was as follows. (Table 1).

1.	**DECREASED PRODUCTION**	
(i)	**Haematological malignancies**	
a.	Acute myeloid leukemia	6
b.	Acute lymphoblastic leukemia	3
c.	Chronic myeloid leukemia in transformation	1
d.	Chronic myeloid leukemia with myelofibrosis	1
(ii)	**Hypoplastic disorders**	
(i).	Aplastic anaemia	4
2.	**INEFFECTIVE HAEMATOPOIESIS**	
(i)	Megaloblastic anaemia	1
(ii)	Myelodysplastic syndrome	2
3.	**CONGESTIVE SPLENOMEGALY WITH HYPERSPLENISM**	1

4. INFECTIONS

(i)	Viral	3
(ii)	Malarial	2
(iii)	Leptospirosis	1
(iv)	HIV with aspergillosis	1

5. CONNECTIVE TISSUE DISEASE

(i)	SLE with pancytopenia	1
(ii)	Juvenile Rheumatoid arthritis with pancytopenia	1
(iii)	SLE with antiphospholipid antibody syndrome and pancytopenia	1
(iv)	Ankylosing spondylitis	1

6. DRUG INDUCED THROMBOCYTOPENIA 2

7. DISSEMINATED INTRAVASCULAR COAGULATION 1

8. MICROANGIOPATHIC HAEMOLYTIC ANAEMIA 1

These cases were then analysed with the entire clinical and laboratory profiles and the possible pathogenesis outlined.

Table1.Distribution of Cases

S.No	Category	No. of Cases
1.	Decreased Production	15
2.	Ineffective haematopoeisis	3
3.	Hypersplenism with congestive splenomegaly	1
4.	Infections	7
5.	Connective Tissue Disease	4
6.	Drug Induced	2
7.	Disseminated Intravascular Coagulation	1
8.	Microangiopathic haemolytic anaemia	1

DISCUSSION

HAEMATOLOGICAL MALIGNANCIES

Thrombocytopenia is well known in acute haematological malignancies due to extensive infiltration of the marrow by blasts replacing normal haematopoietic elements.[31].Our study also showed a significant reduction in megakaryocytes in the marrow in all the cases of acute leukaemia. Our study included 6 cases of AML,3 cases of ALL (Figs 1,2,3,4)

One case of AML M5 in addition showed dysplastic megakaryocytes in the marrow and a significant prolongation of PT and APTT.

The dysplasia producing an ineffective megakaryopoiesis and increased coagulation parameters with gastrointestinal bleeding suggestive of an underlying disseminated intra vascular coagulation could be additional contributory factors to the thrombocytopenia.

Further this patient was on the 4th cycle of chemotherapy with a possible drug induced myelosuppression.

Another case of AML presented as pancytopenia with prolonged APTT. The possibility of myelodysplastic syndrome converting to AML was considered.[32] The patient had a history of native medicine intake the same probably contributing to the myelodysplasia and hypoplastic kidney probably chronic pyelonephritis.

One case of ALL had a significant elevation of LDH with a prolongation of PT and APTT. He was partially treated outside. The possibility of tumour lysis syndrome with DIC was considered.

One patient presented with pancytopenia.[33] with bone marrow showing lymphoblasts and FNAC of cervical node suggesting a lymphoproliferative disorder.

Two cases of CML presented with thrombocytopenia.

One patient had epistaxis, arthralgia and fever, a leucoerythroblastic blood picture, 18% myeloblasts, bone marrow showing 42% myeloblasts, dysplasia and occasional hemo phagocytosis. Increased reticulo endothelial cells,prolonged PT, reduced fibrinogen and increased LDH were also observed.

Apart from the conversion of CML to AML accounting for the thrombocytopenia, the other possibilities considered were:

1. myelodysplastic syndrome with ineffective megakaryopoiesis

2. haemophagocytic histiocytosis as evidenced by increased reticulo endothelial cells and haemophagocytosis in the marrow, increased prothrombin time, decreased fibrinogen and increased LDH.

The second case of CML presented with pancytopenia. She was on treatment with Hydroxyurea and Busulphan based on the availability. The bone marrow aspiration and biopsy were repeated in view of the persistent thrombocytopenia.Marrow showed extensive fibrosis with a few clusters of megakaryocytes. The following possibilities were considered.

a. drug induced fibrosis

b. evolving megakaryoblastic leukemia

HYPOPLASTIC DISORDERS

We had 4 cases of aplastic anaemia in our study. One patient had an absent left kidney and a scaly skin with mild dystrophy of toe nails suggestive of possible dyskeratosis congenita.

INEFFECTIVE HAEMATOPOIESIS

Although a common disorder, our study included only one case of megaloblastic anaemia in a young female who responded well to B12 and folate, ineffective megakaryopoiesis being the main contributor to thrombocytopenia.

There were two cases of primary MDS in this study, both patients being symptomatic for the pancytopenia including thrombocytopenia. Marrow showed trilineal dysplasia, ineffective haematopoiesis contributing to thrombocytopenia.In addition dysplasia was noted in 2 denovo acute leukaemia and CML in blast crisis. Dysplasia (secondary) was also noted in two cases of connective tissue disorder,one drug induced thrombocytopenia and a patient with HIV.

Our study had only one case of hypersplenism with splenic sequestration ie a case of decompensated liver disease with portal hypertension and congestive splenomegaly. Hepatotropic viral markers were negative.and patient also had GradeIII oesophageal varices, decreased fibrinogen ie 112 mg/dL and ascites.Hepatic coagulopathy was considered as an additional factor to thrombocytopenia.

INFECTIONS

Four cases of acute febrile illness with thrombocytopenia were included in the study. 2 were positive for Dengue IgG, IgM.Peripheral destruction of platelets with cross reacting antibodies is the common pathogenetic mechanism for thrombocytopenia[33]. One patient had in addition an increased LDH with marrow showing haemophagocytosis with a possibility of sequestration of platelets in the macrophages as an additional contribution to thrombocytopenia. One patient had decreased megakaryocytes in the marrow and reactive lymphocytes in smear (Fig.5).The third case of acute febrile illness presented with a prolonged pancytopenia with the marrow appearing cellular with partial maturation arrest in granulocytic series, a few megakaryocytic bare nuclei and haemophagocytosis. With the APTT prolongation, clinical features of bleeding and evidence of haemophagocytosis the possibility of virus associated macrophage activation was considered[25,26].

The fourth case also presented with an acute febrile illness and was MSAT positive for Leptospirosis. In addition to immune mediated platelet destruction, inhibited platelet production was considered in the pathogenesis of thrombocytopenia.

Two cases of malaria P.vivax infestation. (Fig.6) presented with thrombocytopenia,the pathogenesis of thrombocytopenia being probably due to [34,16] :

a) splenic sequestration and enhanced macrophage activity.

b) activation of platelets by haemolysed red cells resulting in DIC.APTT was prolonged in one patient

c) Quinine and sometimes chloroquine are sometimes known to cause thrombocytopenia.

There was no evidence of renal dysfunction in both these cases.

We had one patient with HIV presenting with thrombocytopenia. Marrow appeared hypocellular with dysplastic changes. The patient also had Aspergillus pneumonia (Fig.7,8).The following contributors to thrombocytopenia were considered:

1) HIV induced marrow changes

2) Drug induced myelopathy

3) Myelodysplasia with ineffective megakaryopoiesis

There was no evidence of sepsis or DIC in this patient

CONNECTIVE TISSUE DISEASE

4 cases of connective tissue disease were included in this study because of possible non immune mechanisms contributing to thrombocytopenia.

1. One case of SLE presenting with pancytopenia, bleeding diathesis, prolonged APTT, low fibrinogen, increased LDH, the marrow showing adequate haematopoietic elements, increased reticuloendothelial cells with haemophagocytosis. She had evidence of E.coli infection at the time of admission. The possibility of macrophage activation syndrome was considered with a good response to appropriate antibiotics and plasmapheresis

2. A case of Juvenile rheumatoid arthritis also presented with features of macrophage activation syndrome with severe pancytopenia and hepatic dysfunction as evidenced by grossly increased serum alkaline phosphatase and coagulation parameters, low fibrinogen

and increased LDH, clinical hepatospenomegaly and generalized lymphadenopathy.

3) We had a third interesting case of a 46/F who presented with thrombocytopenia and thrombosis.ANA and ACLA were positive. Hams test was negative. She was on steroids and Acitrom. In 6 months, she presented with an abdominal wall hematoma with a grossly prolonged APTT and persistent thrombocytopenia. The peripheral blood showed circulating normoblasts and occasional red cell fragments. The increased APTT with a fairly normal PT and deep seated intramuscular bleeding suggested possibility of acquired antibodies to coagulation factors. The thrombocytopenia, apart from the immune mediated platelet destruction, could be aggravated due to dysplasia in megakaryocyte series

4) One case of ankylosing spondylitis, a young male presented with anaemia and thrombocytopenia with a prolonged APTT. He presented with mild splenomegaly and was on NSAID for spondyloarthropathy.Marrow appeared hypercellular with increased megakaryocytes with micromegakaryocytes, moderate myeloid hyperplasia,increased reticuloendothelial cell activity.The following possibilities were considered:

1) Increased disease activity as evidenced by increased reticuloendothelial cells,increased ESR, prolonged APTT and low normal fibrinogen and macrophage engulfment of platelets.

2) NSAID induced peripheral destruction due to cross reacting antibodies and myelopathy resulting in dysplasia/suppressions.

Drug induced Thrombocytopenia:

1) NSAID induced thrombocytopenia included one case of ankylosing spondylitis (Vide supra).

2) A case of tuberculosis on class I Anti tuberculous therapy for 2 months which included Rifampicin developed thrombocytopenia. Marrow evaluation showed decreased megakaryocytes and no granuloma or fibrosis. The patient was subsequently put on a regime without Rifampicin and platelet counts gradually recovered [35,36,37.]

DISSEMINATED INTRAVASCULAR COAGULATION:

A 52 year old male on treatment for non small cell lung carcinoma with a positive cytology in pleural and ascitic fluid and endobronchial biopsy (Fig. 9) developed a bleeding diathesis while on therapy. The complete blood count showed pancytopenia, increased coagulation parameters low normal fibrinogen, increased fibrin degradation products and D-dimer, increased LDH. The marrow aspirate was hypocellular and

the trephine biopsy showed necrotizing granulomatous inflammation negative for AFB (The patient was however on ATT before the diagnosis of malignancy).The cause of thrombocytopenia in this patient could be attributed to

1. Marrow infiltration by necrotizing granulomata ? Tuberculosis?Carcinomatosis.

2. Increased coagulation parameters and LDH with a d-dimer increase – Disseminated carcinoma with a chronic consumption.

RED CELL FRAGMENTATION:

A 28 year old male with a prosthetic cardiac valve for Rheumatic heart disease for over 20 years presented with fever, breathlessness and bleeding gums. Labroatory data showed a severe thrombocytopenia, low normal fibrinogen and increased LDH. The marrow showed myeloid hyperplasia with adequate megakaryocytes with peripheral smear showing mild anisocytosis with occasional fragmented RBC.USG showed minimal splenomegaly.

The possibility of :

1. Chronic red cell fragmentation with underlying subclinical platelet consumption compounded by

2. A subsequent infection (infective endocarditis) resulting in severe thrombocytopenia and clinical bleeding.

The analysis of 33 cases of thrombocytopenia revealed an interesting mix of etiopathogenetic factors. With the limited laboratory facilities it was possible to deduce to a reasonable level the pathogenetic mechanisms which in many cases were confirmed by response to treatment on follow up of patients.

SUMMARY AND CONLUSION

Thrombocytopenia with or without bleeding manifestations is a common problem in general clinical practice. To treat or not to treat and how to treat would depend a lot on etiopathogenesis, of this problem. As pathologists, it is for us to highlight this point in order to help the clinician manage the patient in a scientific and beneficial manner. As we have seen, supportive parameters like a good peripheral smear examination, coagulation profile, comprehensive marrow evaluation and certain biochemical parameters with the clinical profile go a long way in narrowing the etiology and maximizing treatment benefits. It should be remembered that all thrombocytopenias are not immune mediated and therefore a presumption to this effect should not be made in the first instance. Also in cases of confirmed immune thrombocytopenia one should have an open mind about other non immune processes creeping into pathogenesis during progression of disease. This is a limited study and evaluation of larger number of patients is therefore necessary for a more comprehensive and comparative analysis.

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38.

KEY TO MASTER CHART

1.	TLC	-	Total Leucocyte count
2.	DC	-	Differential count
3.	RBC count	-	Red blood cell count
4.	Hb	-	Haemoglobin
5.	HCT	-	Haematocrit
6.	MCV	-	Mean corpuscular volume
7.	MCH	-	Mean corpuscular Haemoglobin
8.	MCHC	-	Mean corpuscular Haemoglobin concentration
9.	RDW	-	Red cell distribution width
10.	ESR	-	Erythrocyte sedimentation rate
11.	Plt count	-	Platelet count
12.	MPV	-	Mean platelet volume
13.	PDW	-	Platelet distribution width
14.	BT	-	Bleeding time
15.	PT	-	Prothrombin time
16.	APTT	-	Activated partial thromboplastin time
17.	LDH	-	Lactate dehydrogenase

18.	LFT	-	Liver function tests
19.	RFT	-	Renal function tests
20.	USG	-	Ultrasonogram
21.	PS	-	Peripheral smear
22.	BM	-	Bone marrow
23.	N	-	Normal
24.	MK	-	Megakaryocyte
25	MB	-	Myeloblast
26.	Hyper	-	Hypercellular
27.	EP dysp	-	erythroid precursors dysplastic
28.	Mo	-	Monoblasts
29.	HM	-	Hepatomegaly
30.	AML	-	acute myeloid leukaemia
31.	SM	-	splenomegaly
32.	HSM	-	hepatosplenomegaly
33.	TB	-	Total bilirubin
34.	AP	-	alkaline phosphatase
35.	dysplMK	-	dysplastic Megakaryocyte
36.	PCP	-	Pancytopenia

37.	fibr	-	fibrinogen
38.	LB	-	lymphoblast
39.	PE	-	pleural effusion
40.	LPD	-	lympho proliferative disorder
41.	U	-	Urea
42.	Cr	-	Creatinine
43.	TCP	-	thrombocytopenia
44.	RE	-	Reticuloendothelial
45.	MDS	-	myelodysplastic syndrome
46.	NB	-	Normoblast
47.	ANA	-	antinuclear antibodies
48.	ACLA	-	anticardiolipin antibody
49.	SLE	-	systemic lupus erythematosis
50.	HP	–	haemophagocytosis
51.	EH	-	erythroid hyperplasia
52.	DCT	-	Direct Coomb's test
53.	RL	–	reactive lymphocyte
54.	P	–	polymorph
55.	CRP	-	C-reactive protein

56.	RA	-	Rheumatoid arthritis
57.	DCLD	-	Decompensated chronic liver disease
58.	dil PV, SV	–	dilated portal vein, splenic vein
59.	NSCLC	-	Non small cell lung carcinoma
60.	FDP	-	fibrin degradation products
61.	DIVC	–	Disseminated intravascular coagulation
62.	Hypo	–	Hypocellular marrow
63.	Ap. An	-	Aplastic anemia
64.	LKA	–	Left kidney absent
65.	LKH	-	Left kidney hypoplastic
66.	FNA	-	Fine needle aspiration
67.	RC	-	Renal cysts
68.	LEB	-	Leucoerythroblastic picture
69.	CML B.C.	-	Chronic myeloid leukaemia blast crisis
70.	LP	-	Lymphocyte preponderance
71.	FC liver	-	Fatty change liver
72.	Ret hge	-	Retinal haemorrage
73.	Hy MDS	-	hypoplastic MDS
74.	Par wall hem	-	Parietal wall hematoma

75.	mic MK	-	micro megakaryocyte
76.	gi plt	-	giant platelet
77.	MKA	-	megakaryocyte adequate
78.	UC	-	Urine culture
79.	E.C.	-	E.coli
80.	Meg Ane	-	Megaloblastic anaemia
81.	MH	-	myeloid hyperplasia
82.	Mil TB	-	military tuberculosis
83.	Rif Ind. TCP	-	Rifampicin induced thrombocytopenia
84.	Asc	-	Ascitis
85.	Den IgG, IgM	-	Dengue IgG, IgM
86.	LCP	-	Leucopenia
87.	Cyt vac	-	cytoplasmic vacuoles
88.	Dys EP	-	dyserythropoiesis
89.	P.Vx TPZ	-	P. Vivax Trophozoite
90.	Meg EP	-	Megaloblastoid Erythroid precursors
91.	BF	-	Band forms